EFFECTS OF NEURAL ACTIVITY ON THE FIREFLY PSEUDOFLASH[1,2]

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The pseudoflash of the adult firefly, first recorded by Snell in 1932, is produced by subjecting the animal to a sudden increase in oxygen concentration after anoxia. A dull, hypoxic glow develops during anoxia and spreads over the entire organ, gains in intensity, then slowly declines to extinction. If air is readmitted at any time during the hypoxic glow, there occurs a brilliant pseudoflash of one second or longer. According to Snell and later, Alexander (1943), the pseudoflash reflects the unimpeded entry of oxygen into the luminous tissue through supposedly valvular tracheal end cells (Dahlgren, 1917), the valves being temporarily inactivated by hypoxia. This theory implies that firefly luminescence is normally oxygen-limited and that the pseudoflash is independent of neural activity.

McElroy and his associates (summarized in McElroy and Hastings, 1955) suggest the possibility of luminescence control by chemical reactions not directly involving oxygen, so that the light organ may be oxygenated at all times. Further, these workers have proposed a flash-generating mechanism by which the nerve impulse brings about release of pyrophosphate which in turn stimulates the light-yielding reaction.

Hastings and Buck (1956) concluded from experiments on decapitated adults, isolated light organ segments and excised photogenic organs, that nerve impulses originating in the central nervous system played no role in the photogenic response to hypoxia since in all these preparations an hypoxic glow developed in low oxygen concentrations and a pseudoflash resulted with readmission of air. They further noted, as had others previously, that pseudoflash and hypoxic glow involved only one or a portion of one segment in some cases, that some animals failed to give either response, and that considerable variation in both responses occurred even within one individual.

The variations of hypoxic response of individual fireflies, ranging from no response during quiescent periods to complete, two-segment responses in actively flashing animals, indicate that factors besides hypoxia play a role in pseudoflash production. A number of observations, previously reported in abstract form (Carlson, 1959), strongly implicate the nervous system. The purpose of this

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report is to present in detail these observations concerning the role of the innervation in pseudoflash and hypoxic glow.

Materials and Methods

Seventy-four adults of two firefly species, *Photinus pyralis* Linn. and *Photuris* sp., were the subjects of this study. Fireflies were exposed to various concentrations of oxygen in nitrogen and to pure nitrogen. The experimental animal was secured ventral side up on a narrow glass spatula provided with platinum stimulating electrodes. The spatula was placed immediately next to the gas inlet in a glass tube one foot long and one centimeter in diameter. A photomultiplier tube (RCA 931-A) and dissecting microscope were positioned above the gas chamber and both were shielded by black cloth from stray light. Gases entered the chamber through a Y-tube provided with stopcocks arranged to permit rapid alternation of two gas mixtures. Gases were prepared from commercial compressed nitrogen and oxygen metered through two-stage reduction valves and calibrated Fischer-Porter flow meters. Mixture composition was checked by gas analysis with a Scholander 0.5-cc. analyser.

In preparation for a pseudoflash one valve was rotated 180° to shunt off the oxygen and admit either pure nitrogen or a nitrogen-oxygen mixture containing

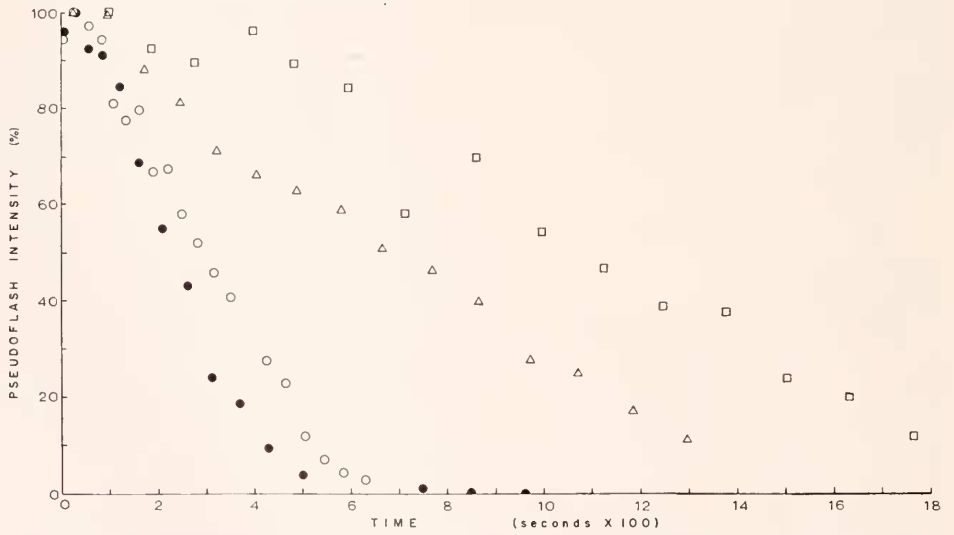

Figure 1. Decrease of pseudoflash intensity in successive pseudoflashes induced at hypoxic glow maximum with recovery periods of constant duration in adult males, *Photinus pyralis*. Ordinate: Pseudoflash intensity in per cent of most intense pseudoflash in each series.

In this and following graphs open circles denote successive pseudoflashes with a constant air recovery period of 10 seconds. Closed circles denote a second run with the same animal immediately after inducing the animal to flash and with a constant air recovery duration of 11 seconds. Squares denote successive pseudoflashes in another animal with a constant air recovery duration of 60 seconds. Triangles denote successive pseudoflashes in a third animal with a constant air recovery duration of 30 seconds. Pseudoflashes in all cases were induced with oxygen concentration of 17.25%. Oxygen concentration during hypoxia was 0.5%.

less than 2.5% oxygen. Rotation of this stopcock also opened a signal circuit. At an appropriate time the same valve was then rotated back 180° which allowed a higher concentration of oxygen to reach the animal suddenly and also closed the signal circuit. The pseudoflash was detected by the photomultiplier, and its output was led to one or both channels of an Offner Dynograph.

Nerve action potentials in the adult light organ during pseudoflash generation were recorded with the animal in a chamber with inlet and outlet tubes and covered with a transparent plastic sheet. Uninsulated platinum recording electrodes were placed on the light organ surface after cuticle removal. Action potentials were led through an AC amplifier to one beam of an oscilloscope. The other beam recorded the photomultiplier output.

Results

1. *Induction of pseudoflashes in Photinus adults*

In actively flashing animals hypoxia initially produces a dark period of five seconds or less, followed by an hypoxic glow, which begins suddenly as a dull glow over the entire lantern, then gradually and uniformly brightens. Pseudoflashes induced after the hypoxic glow develops are of high intensity. In quiescent, non-flashing adults either no hypoxic glow and pseudoflash can be induced even with prolonged hypoxia, or a very dull, spotty, hypoxic glow develops after a dark period lasting for minutes. The subsequent pseudoflash is greatly reduced as compared with those induced in individuals with bright hypoxic glows. If the quiescent animal is first stimulated to flash, its capacity for developing a bright hypoxic glow and pseudoflash is greatly enhanced.

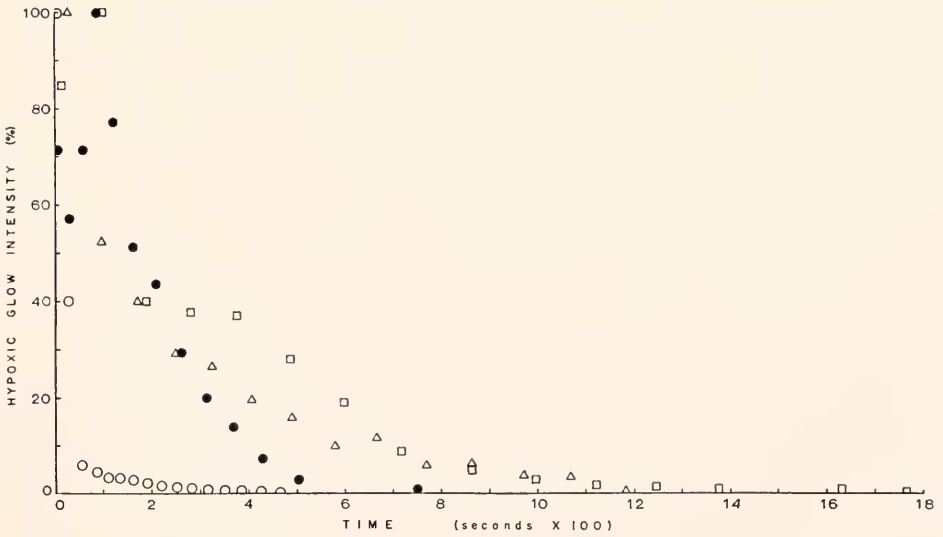

Figure 2. Decrease of hypoxic glow maximum intensity with successive pseudoflashes and with recovery periods of constant duration in adult males, *Photinus pyralis*. Ordinate: Hypoxic glow intensity in per cent of most intense hypoxic glow in each series.

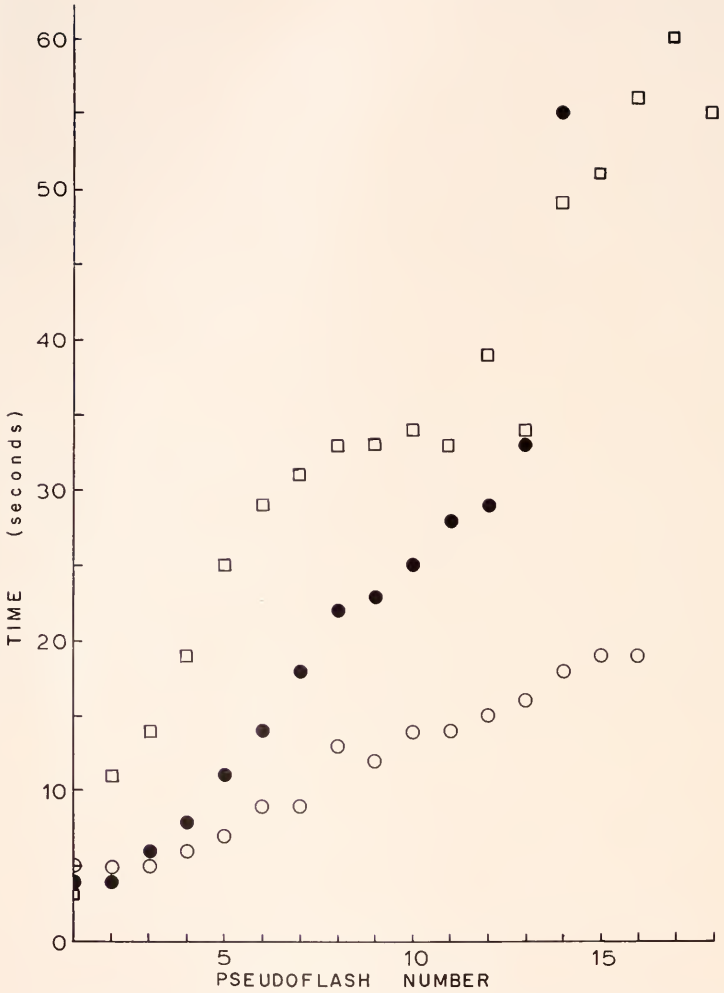

FIGURE 3. Increase in duration of hypoxia prior to the onset of the hypoxic glow in successive pseudoflashes in adult males, *Photinus pyralis*. Ordinate: Hypoxic duration prior to hypoxic glow onset in seconds.

In spontaneously flashing *Photinus* adults a large number of successive pseudoflashes of high intensity can be induced. However, quiescent animals exhibit a characteristic series of changes during successive pseudoflashes which strongly suggest neural involvement. If such animals are first induced to flash spontaneously, then exposed to a given hypoxic oxygen concentration during attainment of hypoxic glow maximum, alternating with fixed periods of exposure to air, the pseudoflashes produced go through the following progressive changes:

(1) Pseudoflash intensity slowly declines (Fig. 1). (2) Hypoxic glow intensity rapidly declines initially, then declines at a slower rate (Fig. 2). (3) The

time required from onset of hypoxia to onset of the hypoxic glow increases (Fig. 3). (4) The time from onset of hypoxia to the attainment of maximal hypoxic glow increases (Fig. 4). (5) Animals that have undergone these changes can be restored to their original condition of intense hypoxic glow and pseudoflash by stimulating mechanically or electrically until they begin to flash spontaneously (Figures 1, 3 and 4; open and closed circles showing two successive runs with the same animal). The rate at which these progressive changes occur is highly variable not only among different individuals but also within the same individual at different times. The graphs indicate the range of effects observed in a total of 30 runs on 13 individuals studied.

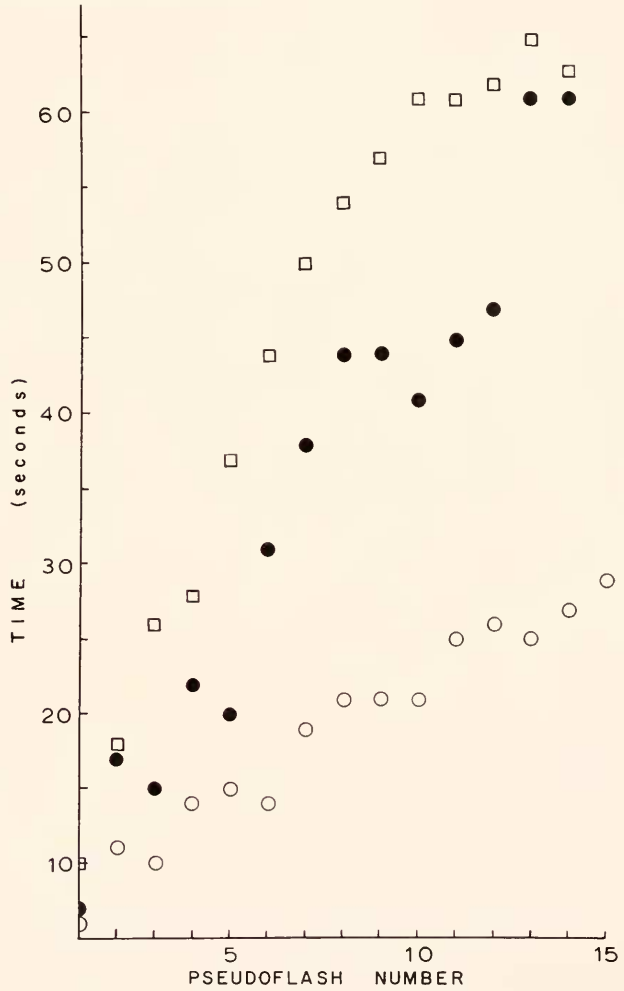

FIGURE 4. Increase in duration of hypoxia prior to attainment of maximum hypoxic glow intensity with successive pseudoflashes in adult males, *Photinus pyralis*. Ordinate: Hypoxic duration prior to hypoxic glow maximum intensity in seconds.

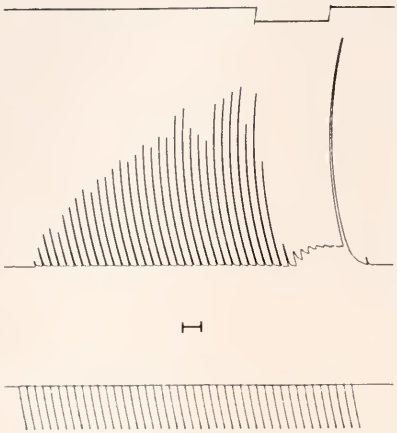

FIGURE 5. Electrically excited neural flashes in air and during hypoxia, followed by a single pseudoflash in an adult male, *Photinus pyralis*. Time axis reads from left to right. Top trace: up, 17.9% oxygen; down, .065% oxygen. Middle trace: light intensity. Bottom trace: stimulus signal. One stimulating electrode in anterior abdomen and other dorsal to light organ through anus. Stimulation: 8 volts, 50-msec. duration, frequency 0.5 per second. Heavy horizontal line represents 5 seconds on this and all subsequent figures except where otherwise noted.

2. *Effects of electrical stimulation on hypoxic glow and pseudoflash*

Electrical stimulation of a firefly in air normally produces a facilitating series of flashes. When stimulation continues into the hypoxic period, flashes diminish and a high intensity hypoxic glow appears (Fig. 5). A pseudoflash can be induced

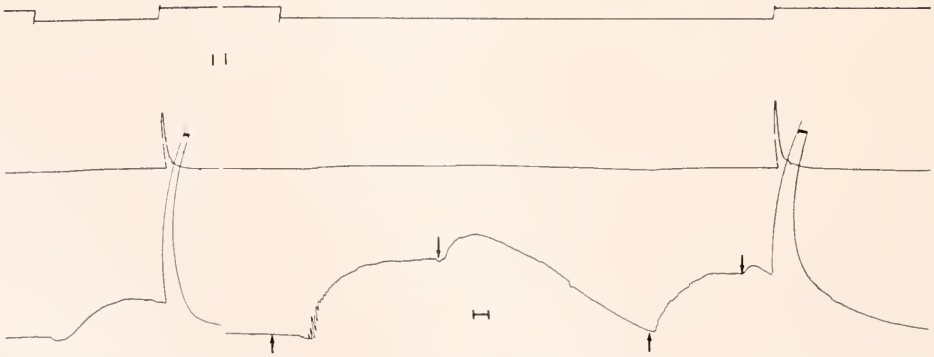

FIGURE 6. Effect of electrical stimulation during hypoxia upon hypoxic glow in an adult male, *Photinus pyralis*. Top trace: up, 17.25% oxygen; down, .05% oxygen. The middle and bottom traces both indicate light intensity, the bottom trace at 10 times the amplification of the middle trace. Stimulating electrode pair in abdomen dorsal to light organ. Stimulation: 5 volts, 50-msec. duration, frequency 1 per second. Short parallel lines indicate a break in the record. In this figure and subsequent figures where used, arrows pointing up indicate stimulus onset and arrows pointing down indicate stimulus cessation. The figure shows first an anoxic sequence without electrical excitation followed by two stimulus trains during hypoxia. In last two episodes glow rose temporarily after stimulation ceased.

as soon as electrically excited flashes undergo an increase in duration, which appears to be characteristic of the hypoxic state. Electrical stimulation during hypoxia increases the rate of hypoxic glow rise and intensity of glow attained (Fig. 6). It will also be noted that when stimulation ceases a rapid brightening usually occurs followed by a decline, an effect which is typically observed. The hypoxic glow was maintained in one animal for 20 minutes by periodic stimulation in 0.6% oxygen; without stimulation it might be expected to persist less than two minutes.

Although prolonged stimulation during hypoxia favors a pseudoflash of increased duration, stimulation, when continued during readmission of air, superimposes no discrete flashes on the pseudoflash rise. However, this may occur during pseudoflash decay as illustrated in Figure 7. Electrical stimulation commonly augments the hypoxic glow by recruiting new, discrete luminescing areas

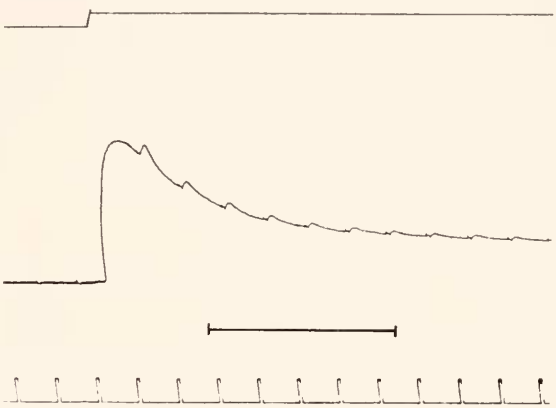

FIGURE 7. Electrical stimulation during readmission of 17.75% oxygen and consequent pseudoflash in an adult male, *Photinus pyralis*. Top trace: up, 17.75% oxygen; down, .05% oxygen. Middle trace: light intensity. Bottom trace: stimulus signal. Stimulating electrode pair placed in sides of abdomen dorsal to light organ. Stimulation: 50 volts, 40-msec. duration, frequency 1 per second. Tiny marks on middle trace most apparent during hypoxia are stimulus artifacts via ground circuit, not light emission.

of the lantern in serial fashion, one or more areas per pulse. The effect is illustrated in Figure 8; each pulse initiates luminescence which covers one additional area completely.

3. *Effects of an anticholinesterase and of denervation on hypoxic glow and pseudoflash*

In order to examine further the role of possible neural activity on the pseudoflash, the effects of injection of an anticholinesterase and of nerve transection on the hypoxic glow and pseudoflash were studied. Eserine, 10^{-4} *M*, in Roeder's saline (Roeder, 1953), injected into the body cavity of the decapitated adult, after removing the tip of the abdomen to permit flow of the solution through the body,

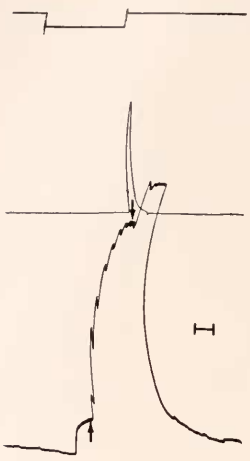

FIGURE 8. Effects of electrical stimulation during rising phase of hypoxic glow in an adult male, *Photinus pyralis*. With each shock hypoxic glow augmented as new areas begin to glow and remain glowing. Same effect can be noted in Figure 6, after first arrow. Top trace: up, 17.25% oxygen; down, .05% oxygen. Middle and bottom traces: light intensity, amplification of bottom trace being 1000 times middle trace. Stimulating electrode pair on right and left sides of seventh abdominal segment dorsal to light organ. Stimulation: 10 volts, 50-msec. duration, frequency 1 per second.

produces nearly continual high intensity scintillation and flashing in air (Case and Buck, 1959). Under these conditions the rate of rise and maximal intensity of hypoxic glow are increased greatly. Pseudoflashes of normal characteristics can be induced after as little as 5 seconds of hypoxia (Fig. 9).

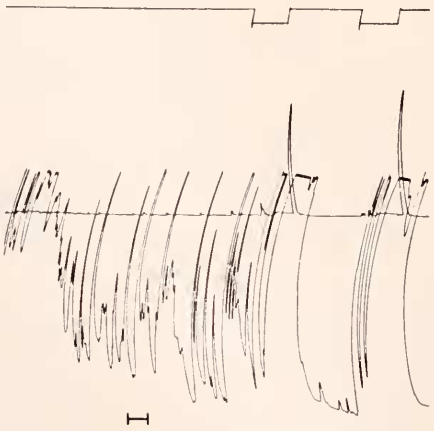

FIGURE 9. Effect of perfusion of 10^{-4} *M* eserine through a decapitated adult male, *Photinus pyralis*. Note the continual scintillation and rapid onset of hypoxic glow. Top trace: up, 17.25% oxygen; down, .05% oxygen. Middle and bottom traces: light intensity, amplification of bottom trace being 200 times middle trace.

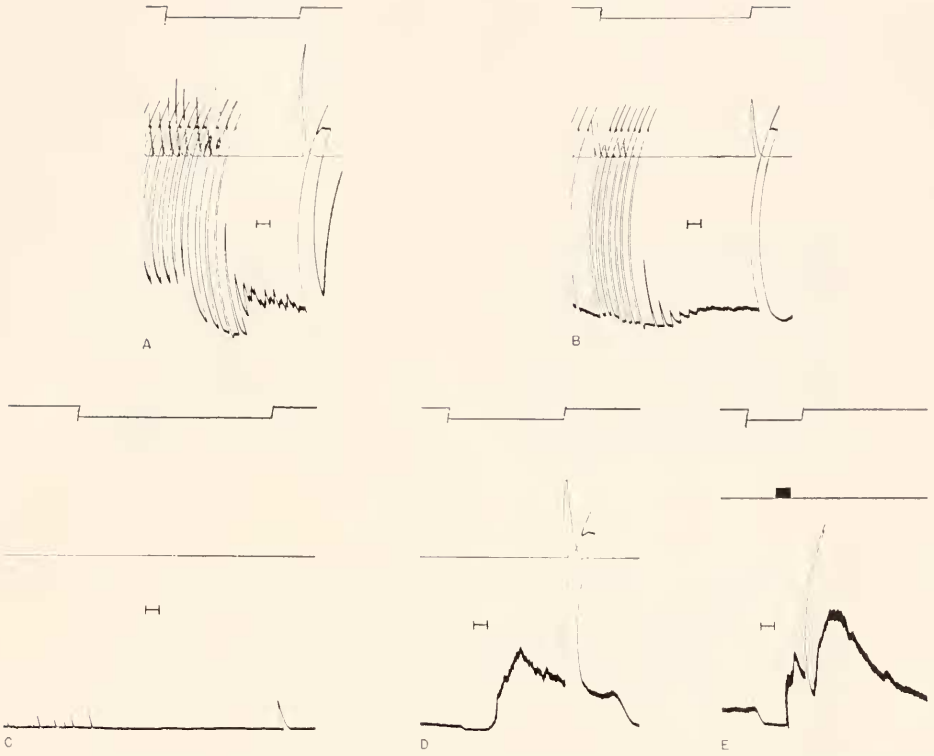

FIGURE 10. Effect of transection of the ventral nerve cord between the sixth and seventh abdominal segments of a female, *Photuris sp.* Except where noted otherwise the following conditions were maintained. Top trace: up, 21% oxygen; down, .05% oxygen. Middle and bottom traces: light intensity except where otherwise noted, amplification of bottom trace being 500 times middle trace. (A) Normal pseudoflash, both sixth and seventh abdominal segments give a total flash. Spontaneous flashing is seen in this figure, as well as in B, during onset of hypoxia. (B) Normal pseudoflash, sixth segment masked. Note the reduction in intensity of the pseudoflash since only the seventh segment is being recorded. (C) Inability to induce pseudoflash in segment 7, 8 minutes after transection, sixth segment masked. Top trace: up, 21.4% oxygen; down, 0.4% oxygen. Light leak from sixth segment appears as small flashes on left, bottom trace. (D) Pseudoflash 20 minutes after transection, seventh segment masked, showing response in sixth segment still normal. Amplification of light signal in bottom trace, 1000 times middle trace. (E) Pseudoflash 45 minutes after transection with stimulation, sixth segment masked. Stimulating electrode pair inserted laterally into the seventh segment dorsal to the light organ. Middle trace: stimulus signal. Bottom trace: light intensity with same amplification as before. Stimulation: 15 volts, 20-msec. duration, frequency 20 per second.

In some instances, transection of nerves supplying the light organ of abdominal segment 7, at the level of the intersegmental membrane between segments 6 and 7, abolishes spontaneous flashing in the seventh segment and leaves unaffected the light organ of segment 6. Gradual reduction of hypoxic glow and pseudoflash intensity occurs in segment 7 until both are completely abolished after two hours, while the response in segment 6 can be maintained by mechanical irritation, eliciting

spontaneous flashes. In other cases, particularly in *Photinus,* the pseudoflash is immediately abolished in segment 7, except in one or two tiny spots which gradually lose the ability to generate pseudoflashes. Stimulation with electrodes placed dorsally in the seventh (denervated) segment partially restores the pseudoflash and hypoxic glow. The hypoxic glow can also be intensified by stimulation during hypoxia as in the normal light organ (Fig. 10).

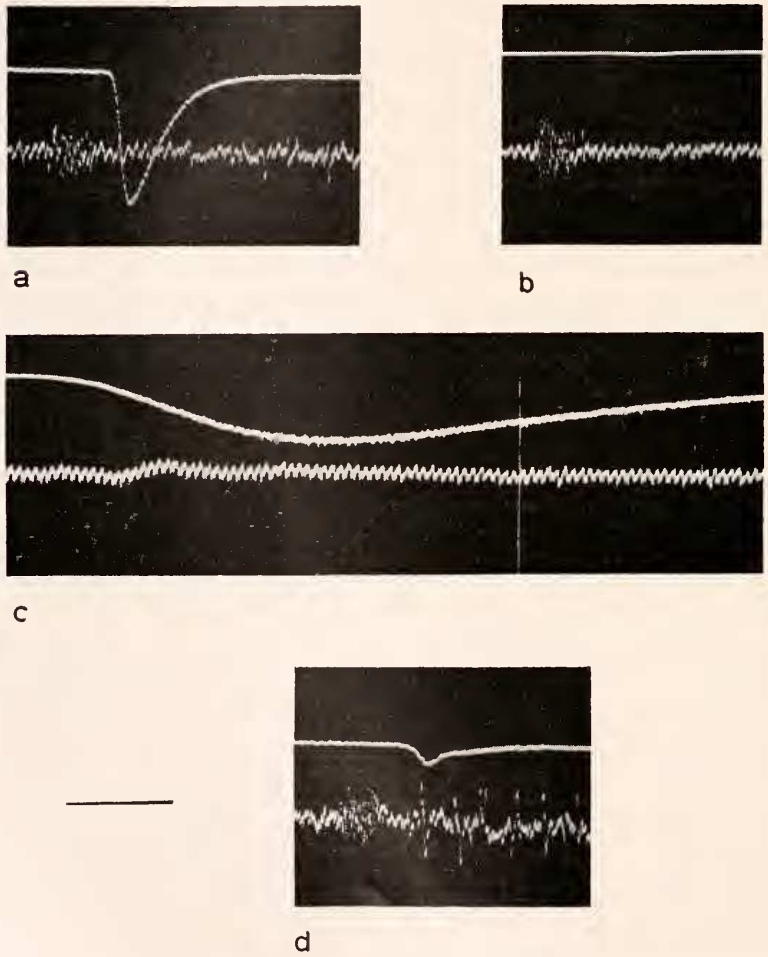

FIGURE 11. Recording of neural action potentials, spontaneous flashes, and pseudoflash in the lantern of an adult male, *Photuris pennsylvanica.* Photomultiplier output on upper trace (increasing intensity down). Action potentials on lower trace. Time base indicated by horizontal, dark line which represents 100 milliseconds. Time base reads from left to right. (a) Spontaneous flash and generating neural burst prior to hypoxia. (b) Extremely reduced spontaneous flash and preceding neural burst after hypoxia onset. (c) Pseudoflash induced with air. Neither neural nor muscle action potentials are seen. (d) Spontaneous flash and preceding neural burst following pseudoflash in air recovery period.

4. *Demonstration of lack of spontaneous neural activity during pseudoflash*

In order to further define the role of the nerves in pseudoflash generation, nerve action potentials were sought during the hypoxic period and pseudoflash by placing recording electrodes on the exposed ventral surface of the light organ. The results are illustrated in Figure 11. The animal was flashing spontaneously prior to the onset of hypoxia and before each flash a discrete burst of neural activity was noted (Fig. 11a). Immediately after onset of hypoxia two flashes of reduced magnitude were observed, each associated with an undiminished train of action potentials (Fig. 11b). Following these flashes no bursts or even isolated nerve impulses could be observed. Although no hypoxic glow was observed, a pseudoflash was produced without associated action potentials (Fig. 11c). Spontaneous flashes and associated neural bursts resumed immediately following the pseudoflash in air (Fig. 11d).

Discussion

These observations on active, quiescent, electrically stimulated and eserine-injected animals, and the results of nerve transection, strongly implicate the innervation in the pseudoflash. Our inability to detect nerve impulses during the hypoxic glow period, however, indicates that individual pseudoflashes in the intact animal can be induced independently of neural activity. One supposition seemingly warranted by the decline of successive pseudoflashes in quiescent animals is that previous neural activity serves as a priming mechanism. A priming activity with regard to spontaneous activity has been suggested by Case and Buck (1959).

The priming mechanism of neural activity on hypoxic glow and pseudoflash can be explained on the basis of the scheme of firefly luminescence developed by McElroy and his associates. Hypoxia may allow substrate accumulation from precursors in the intact animal just as it does *in vitro* as shown by Hastings, McElroy and Coulombre (1953), who were able repeatedly to induce flashes similar to pseudoflashes by alternating hypoxic and aerobic conditions until surface denaturation of the enzyme occurred. The reduction of intensity in successive pseudoflashes in our experiments would represent, according to this scheme, accumulation of an inhibitory complex. When pseudoflashes can no longer be induced, presumably all the enzyme is inhibited. As McElroy and Hastings (1955) speculated, one result of neural stimulation might be release of inorganic pyrophosphate which on the basis of *in vitro* observations would free the active enzyme and result in the ability to again induce high intensity pseudoflashes.

If nerves operate in fact as a priming mechanism by mediating destruction of an inhibitor, a number of observations of previous investigators could be explained. This action might explain why it is difficult or impossible to elicit pseudoflashes in quiescent animals since in them the active enzyme would be completely inhibited. This suggests that during the daily non-flashing periods the light organ would contain large amounts of this inhibitor, and neural activity would be low or absent. The great deal of areal variation observed in the pseudoflash response may simply be the result of spotty neural priming. This is supported by the observation that a total organ pseudoflash is usually obtained in actively flashing animals and by the observations that electrical stimulation during

hypoxia in quiescent animals commonly augments the hypoxic glow by recruiting new, discrete luminescing areas (Fig. 8).

Summary

1. Hypoxic glows and pseudoflashes were studied in adults of the lampyrid fireflies, *Photinus pyralis* Linn. and *Photuris* sp.

2. In spontaneously flashing *Photinus* adults a large number of successive pseudoflashes of high intensity can be induced. Induction of pseudoflashes in quiescent animals results in decline of successive pseudoflash and hypoxic glow intensities, and increase in the time required not only for hypoxic glow onset but also for development of maximal hypoxic glow intensity.

3. Electrical stimulation during hypoxia increases the rate of hypoxic glow rise, commonly by recruiting new luminescing areas within the lantern, and can maintain the hypoxic glow beyond the normal period. Electrical stimulation when continued during readmission of air superimposes flashes on the pseudoflash decay phase only.

4. Perfusion of the body cavity with 10^{-4} *M* eserine enhances the ability to induce high intensity pseudoflashes and hypoxic glows.

5. Transection of the nerves supplying the seventh abdominal segment reduces the ability of that segment to produce pseudoflashes, while pseudoflashes can be obtained from that portion of the lantern occupying the sixth segment. Electrical stimulation of the seventh segment after nerve transection can partially restore its pseudoflash capability.

6. No nerve impulses were observed during the hypoxic interval immediately prior to and during induction of the pseudoflash although neural bursts and their associated flashes were noted before and after hypoxia and during its early period.

7. The observations implicate the innervation in the pseudoflash. A possibility suggested is that previous neural activity serves as a priming mechanism by mediating the removal of a chemical inhibitor.

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